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# A New Synthetic Retinoid, E-5166, Augments Epidermal Beta-adrenergic Adenylate Cyclase Response

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Effects of a new synthetic retinoid, 3, 7, 11, 15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid (E-5166), on the cyclic AMP system of pig skin epidermis was investigated. When pig skin slices were incubated in vitro for 24 h, the beta-adrenergic adenylate cyclase response (epinephrine-induced cyclic AMP accumulation) was decreased. The addition of E-5166 in the incubation medium resulted in an increase of this receptor response of epidermis. On the other hand, histamine-induced cyclic AMP accumulations were decreased by the E-5166 treatment. The effect of E-5166 was concentration-dependent; the maximal effect was observed at 50–100 μM. Ro 10-1670 (an active derivative of Ro 10-9359 (etretinate)) is known to have similar beta-adrenergic augmentation effect. The simultaneous addition of E-5166 and Ro 10-1670 at their optimal concentrations resulted in neither additive nor synergistic effect on the epinephrine-induced cyclic AMP accumulations. There was no significant difference in cyclic AMP phosphodiesterase activity between control and E-5166-treated epidermis. These data indicate that pig skin epidermal adenylate cyclase responses are modulated by E-5166 probably through the same mechanism induced by other retinoids. *Key words: Cyclic AMP; Epidermis.* (Received April 10, 1985.)

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Cyclic nucleotides have been supposed to be important modulators of epidermal keratinocyte proliferation and differentiation, the metabolism of which is altered in various pathologic conditions of epidermis (1). In psoriasis, for example, the involved epidermis is characterized by a relatively specific defect of beta-adrenergic adenylate cyclase system (2) and also by an increased cyclic nucleotide phosphodiesterase activity (3). Since these changes have been observed in experimentally-induced hyperproliferative conditions of epidermis (4, 5), it has been suggested that the resultant markedly defective beta-adrenergic response might be a concomitant feature of epidermal hyperproliferation (6). Interestingly, agents which inhibit epidermal keratinocyte proliferation and have beneficial effects on psoriasis (such as glucocorticoids, colchicine, and protein synthesis inhibitors) have been known to augment the beta-adrenergic adenylate cyclase response of epidermis in vitro (7–9), with a possible participation of the inhibition of the phosphodiesterase activity (8, 9).

Vitamin A and its analog (retinoids) are other important modulators of keratinocyte proliferation and differentiation (10). Recently the efficacy of synthetic retinoids in the management of psoriasis has been demonstrated in a large number of clinical trials (11). We have shown that retinoids (all-trans-retinoic acid, and Ro 10-1670, an active derivative of Ro 10-9359 (etretinate)) have similar augmentation effects on the beta-adrenergic respone of epidermis in vitro (12). Since there are many types of retinoids with different structural formulations (13), the question whether the beta-adrenergic augmentation is a generalized feature of these compounds remains to be elucidated, which might be important in terms of their so-called antipsoriatic activity.

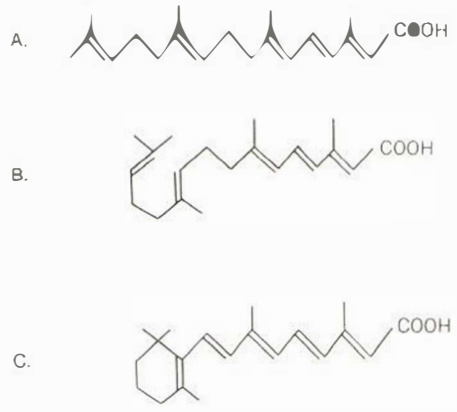


*Fig. 1.* Structural formula of E-5166 (3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid) in two different formulations (A & B). Note the structural similarity to all-trans-retinoic acid (C).

A new retinoid analog, 3,7,11,15-tetramethyl-2,4,6,10,14-hexadecapentaenoic acid (E-5166) (Fig. 1) has recently become available for clinical testing. This new polyprenoic acid compound reveals antitumor activity for experimental skin tumors (14), and clinical efficacy for psoriasis has been shown in a pilot study (Kukita, Hirone, et al. (to be submitted)). In this communication, we investigated the effect of this new retinoid on the cyclic AMP system of pig skin epidermis.

## MATERIALS AND METHODS

The experimental procedures used were the same as those previously described (8, 9). Domestic pigs weighing about 10–15 kg were intraperitoneally anaesthetized with nembutal (dose, 30 mg/kg; Abbott Laboratories, North Chicago, Ill, USA). After fifteen minutes, skin slices were taken from the backs of the pigs with a Castroviejo keratotome (Storz Instrument, St. Louis, MO, USA) adjusted to a 0.2 mm setting. Each skin slice was cut into squares (5×5 mm), washed three times in RPMI 1640 medium

Table 1. *Effects of E-5166 and Ro 10-1670 on the adenylate cyclase responses*

| | Cyclic AMP pmoles/mg protein | | |
|---|---|---|---|
| | No addition | Epinephrine | Histamine |
| 1. *0-time experiments* | | | |
| Control | 1.3±0.1 | 7.5±0.4 | 43.9±2.1 |
| E-5166 | 1.2±0.1 | 7.9±0.6 | 52.4±6.8 |
| Ro 10-1670 | 1.2±0.1 | 6.8±0.5 | 48.5±3.3 |

Skin squares were preincubated for 15 min to standardize the cyclic AMP level (15). Then the squares were incubated with 50 μM epinephrine, or 1 mM histamine for 5 min in the presence or absence of 100 μM E-5166 or 10 μM Ro 10-1670. Results are the means ± SE ($n$=4)

| | | | |
|---|---|---|---|
| 2. 24-h incubation experiments | | | |
| Control | 1.2±0.1 | 2.0±0.2 | 76.2±5.2 |
| E-5166 | 1.2±0.1 | 8.1±0.4* | 25.5±3.0* |
| Ro 10-1670 | 1.2±0.1 | 7.6±0.6* | 10.0±0.8* |

Skin squares were incubated with 100 μM E-5166 or 10 μM Ro 10-1670 for 24 h. After the incubation, skin squares were incubated with 50 μM epinephrine or 1 mM histamine as described in the text. Results are the means ± SE ($n$=4). * $p<0.01$ compared with the control

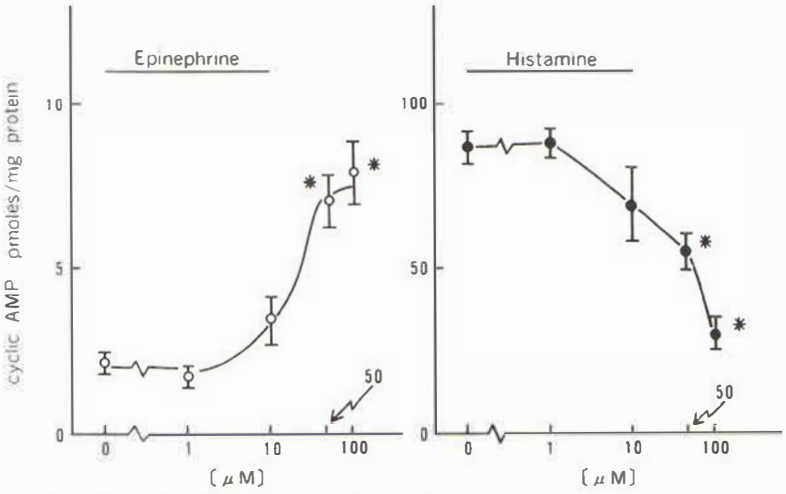


*Fig. 2.* Effects of various concentrations of E-5166 on the beta-adrenergic, and histamine responsiveness of the skin. Pig skin squares were incubated with various concentrations of E-5166. E-5166 was dissolved in DMSO and the final concentration of DMSO in the incubation medium was 0.5 % (v/v). Control medium contained 0.5 % DMSO alone. At 24 h incubation, skin squares were then incubated with adenylate cyclase stimulators as described in the text. Data are the means ± SE of 4 independent experiments. * $p<0.01$ compared with control skin response.

and floated with the keratin layers uppermost in 10 ml RPMI 1640 medium with added antibiotics (100 units/ml penicillin, 0.1 mg/ml streptomycin and 0.25 μg/ml fungizone) and various chemicals to be tested. The incubations were done without the addition of serum at 37°C in an atmosphere of 5 % $CO_2$ in air. Retinoids were dissolved in DMSO, and the final concentration of DMSO was 0.5 % v/v, unless otherwise stated. As a control, DMSO alone was added to the medium. After a long term (up to 24 h) incubation, the skin squares were transferred and floated in fresh RPMI 1640 medium at 37°C for cyclic AMP accumulation studies. Skin squares were preincubated for 15 min at 37°C in a new RPMI 1640 medium, and were then incubated with 50 μM epinephrine or 1 mM histamine for 5 min. After the incubation, the skin squares were quickly frozen between two plates of dry ice. After partial purification by the method of Yoshikawa et al. (15), the cyclic AMP content in these skin squares was measured by radioimmunoassay using a Yamasa cyclic AMP assay kit (Yamasa Shoyu, Tokyo).

The cyclic AMP phosphodiesterase activities in skin squares were measured by the method as

Table II. *Effects of combinations of drugs on the beta-adrenergic adenylate cyclase responses*

| | Cyclic AMP pmoles/mg protein | |
|---|---|---|
| | No addition | Epinephrine |
| Control | 1.2±0.1 | 2.9±1.0 |
| E-5166 | 1.1±0.1 | 9.3±1.0** |
| Ro 10-1670 | 1.1±0.1 | 8.8±0.9** |
| E-5166 + Ro 10-1670 | 1.0±0.1 | 7.4±0.7* |
| Colchicine | 1.3±0.1 | 23.3±2.9** |
| E-5166 + Colchicine | 1.2±0.1 | 36.2±4.0** |

Pig skin squares were incubated with various chemicals for 24 h, and were then incubated with 50 μM epinephrine for 5 min. Data are the means ± SE ($n=4$). The concentrations of each chemical were E-5166 (100 μM); Ro 10-1670 (10 μM); colchicine (1 μM). * $p<0.05$ compared with the control. ** $p<0.01$ compared with the control.

Table III. *Cyclic AMP phosphodiesterase activities in control and E-5166-treated skin*

| | Phosphodiesterase activity (pmoles/min/mg protein) | |
|---|---|---|
| | low Km | high Km |
| Control | 15.1±2.3 | 275±36 |
| E-5166-treated | 13.1±0.9 | 244±26 |

Skin squares were incubated in RPMI 1640 medium from 24 h with and without 100 μM E-5166. After the incubation, two skin squares were frozen between two plates of dry ice. Results are the means ± SE ($n$=4).

previously described (8, 9). The substrate cyclic AMP concentrations for low and high Km enzymes were 0.75 μM and 102 μM respectively. Protein concentration was measured by the method of Lowry et al. (16).

E-5166 and Ro 10-1670 (an active derivative of Ro 10-9359 (etretinate) were generous gifts from Eizai Co. (Tokyo, Japan) and Nippon Roche K.K. (Tokyo, Japan) respectively. RPMI 1640 medium was purchased from Biken (Osaka, Japan). Epinephrine was obtained from Daiichi Pharmaceutical (Tokyo, Japan). The penicillin-streptomycin-fungizone mixture was obtained from M.A. Bioproducts (Walkersville, MD, USA). All other chemicals were obtained from Nakarai Chemicals (Kyoto, Japan).

## RESULTS

The direct addition of E-5166 or Ro 10-1670 in the incubation medium for cyclic AMP accumulations had no effect on the epinephrine-induced or histamine-induced cylic AMP accumulations of pig skin epidermis (Table I, 0-time experiments). However, when pig skin squares were incubated with E-5166 or Ro 10-1670 for 24 h, there was a marked difference in epinephrine-induced, and histamine-induced cyclic AMP accumulations between control and retinoid-treated skin (Table I, 24 h incubation experiments). The epinephrine-induced cyclic AMP accumulation of the control skin decreased during the 24h incubation, whereas those of retinoid-treated skin remained high. On the other hand, histamine-induced cyclic AMP accumulations were markedly decreased by the treatment with retinoids for 24 h. There was no significant difference in the basal levels of cyclic AMP between control and retinoid-treated skin after 24 h incubation.

The effect of E-5166 was concentration-dependent and apparently the maximal effect was observed at 50–100 μM concentration. No effect was observed at 1 μM concentration (Fig. 2). The addition of E-5166 and Ro 10-1670 at their optimal concentrations in the incubation medium resulted in neither additive nor synergistic effect on the epinephrine-induced cyclic AMP accumulations (Table II). On the other hand, the addition of E-5166 and colchicine at their optimal concentrations resulted in the more marked (additive or synergistic) effect than the single addition of each chemical (Table II), suggesting that they probably work through a different mechanism. There was a slight decrease in the phosphodiesterase activity by the E-5166 treatment for 24 h; the differences, however, were statistically not significant.

## DISCUSSION

In a preceding report (12), we have shown that physiologic and synthetic retinoids such as all-trans-retinoic acid, Ro 10-1670 have augmentation effects on the epinephrine-induced cyclic AMP accumulation of pig skin epidermis in vitro. It was also shown that the

retinoids probably work through a different mechanism from that stimulated by glucocorticoids or colchicine, which are two known drugs with similar beta-adrenergic augmentation effects (7, 8).

E-5166 is a new synthetic retinoid recently developed in Japan. In the course of the clinical trial of the drug for psoriasis, it was found that E-5166 required higher dose for the effect than Ro 10-9359 (etretinate), and that mucocutaneous toxic effects such as scaling and cheilitis are not the feature of E-5166. Our data indicate that despite these differences, E-5166 augmented the beta-adrenergic response (and decreased the histamine response) of epidermis as well as other physiologic and synthetic retinoids (Table I and (12)). Thus the modulation of the adenylate cyclase response seems to be a generalized feature of retinoid effects so far studied. Since the adddition of both E-5166 and Ro 10-1670 in the long term incubation medium resulted in neither additive nor synergistic effect on the beta-adrenergic response (Table II), these chemicals seem to work at the same site possibly through the cytoplasmic retinoic acid binding protein (17) or F-type retinoid binding protein (18). Consistent with the requirement of higher dose in the clinical situation, E-5166 required higher concentration for the beta-adrenergic augmentation than Ro 10-1670. The decreased histamine response at higher concentration of E-5166 might indicate toxic effect of this compound in a long term incubation in vitro. The beta-adrenergic augmentation effect, however, was not affected at this higher concentration (Fig. 2).

Although the effect of retinoids on epidermal keratinocyte proliferation is controversial (10, 19), our results are interesting in terms of clinicl efficacy of E-5166 (and other retinoids) on the psoriatic hyperproliferative epidermis. As was mentioned above, psoriasis-involved epidermis has been characterized by the defective beta-adrenergic response (2), and the increased phosphodiesterase activity (3). Increased histamine-adenylate cyclase response is another feature of psoriatic hyperproliferative epidermis (2). Apparently retinoids (including E-5166) seem to have reversing effects on the cyclic AMP system of epidermis in vitro; i.e. increasing the beta-adrenergic response (Table I, Fig. 2), decreasing the histamine response (Table I, Fig. 2), and decreasing the phosphodiesterase activity (Table III). At present we have no explanation how these changes are associated with the physiologic/pharmacologic effects of retinoids on the keratinocyte biological activity, or with the clinical efficacy of retinoids on the psoriatic hyperproliferative epidermis. The effect of cyclic AMP *per se* on the epidermal keratinocyte proliferation is conflicting depending on the culture conditions in vitro (20). Nevertheless, the relation between the epidermal adenylate cyclase responsiveness and the epidermal proliferative activity seems to be marked (12), lending support for the hypothesis that retinoids (as well as glucocorticoids and colchicine) might reveal their clinical efficacy on the psoriatic hyperproliferative epidermis through the modulation of epidermal cyclic AMP system. Whether similar modulation mechanism by these chemicals can be observed in psoriatic human epidermis requires further study for clarification.

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## REFERENCES

1. Iizuka H, Ohkawara A, Ishibashi Y. Human skin epidermal adenylate cyclase systems: Defective beta-adrenergic responsiveness in the involved epidermis of Darier's disease. In: Seiji M, Bern-

stein IA, eds. Normal and abnormal epidermal differentiation. Tokyo: University of Tokyo Press, 1983: 45–58.
2. Iizuka H, Adachi K, Halprin KM, Levine V. Cyclic AMP accumulation in psoriatic skin: Differential responses to histamine, AMP, and epinephrine by the uninvolved and involved epidermis. J Invest Dermatol 1978; 70: 250–253.
3. Iizuka H, Adachi K, Halprin KM, Levine V. Cyclic nucleotide phosphodiesterase in the uninvolved and involved skin of psoriasis. J Invest Dermatol 1978; 70: 246–249.
4. Mufson RA, Fisher SM, Verma AK, Gleason GL, Slaga TJ, Boutwell RK. Effects of 12-o-tetradecanoyl phorbol-13-acetate and mezerein on epidermal ornithine decarboxylase activity, isoproterenol-stimulated levels of cyclic adenosine 3′:5′-monophosphate, and induction of mouse skin tumors in vivo. Cancer Res 1979; 39: 4791–4795.
5. Verma AK, Froscio M, Murray AW. Croton oil- and benzo(a)pyrene-induced changes in cyclic adenosine 3′:5′-monophosphate and cyclic guanosine 3′:5′-monophosphate phosphodiesterase activities in mouse epidermis. Cancer Res 1976; 36: 81–87.
6. Iizuka H, Umeda K, Koizumi H, Aoyagi T, Miura Y. Epinephrine-induced cyclic AMP accumulation in the psoriatic epidermis. Acta Derm Venereol (Stockh) 1981; 61: 391–395.
7. Iizuka H, Ohkawara A. Effects of glucocorticoids on the beta-adrenergic adenylate cyclase system of pig skin. J Invest Dermatol 1983; 80: 524–528.
8. Iizuka H, Kishiyama K, Ohkuma N, Murata H, Ohkawara A. Colchicine-induced alteration of hormone-stimulated cyclic AMP synthesis in pig skin (epidermis). J Invest Dermatol 1984; 82: 357–361.
9. Iizuka H, Kishiyama K, Ohkuma N, Ohkawara A. Modulation of pig epidermal adenylate cyclase responses by protein synthesis inhibitors: Its relation to glucocorticoid and colchicine effects. Arch Dermatol Res (in press).
10. Marcelo CL, Madison KC. Regulation of the expression of epidermal keratinocyte proliferation and differentiation by vitamin A analogs. Arch Dermatol Res 1984; 276: 381–389.
11. Tsambaos D Orfanos CE. Antipsoriatic activity of a new synthetic retinoid. Arch Dermatol 1983; 119: 746–751.
12. Iizuka H, Ohkuma N, Ohkawara A. Effects of retinoids on the cyclic AMP system of pig skin epidermis. J Invest Dermatol (in press).
13. Bollag W. The development of retinoids in experimental and clinical oncology and dermatology. J Am Acad Dermatol 1983; 9: 797–805.
14. Muto Y, Moriwaki H. Antitumor activity of vitamin A and its derivatives. J Natl Cancer Inst 1984; 73: 1389–1393.
15. Yoshikawa K, Adachi K, Halprin KM, Levine V. Cyclic AMP in skin: Effects of acute ischemia. Br J Dermatol 1975; 92: 249–254.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265–275.
17. Elias PM, Williams ML. Retinoids, cancer, and the skin. Arch Dermatol 1981; 117: 160–180.
18. Muto Y, Moriwaki H, Omori M. In vitro binding affinity of novel synthetic polyprenoids (polyprenoic acids) to cellular retinoid-binding proteins. Gan 1981; 72: 974–977.
19. Aoyagi T, Kamigaki K, Kato N, Fukaya T, Iizuka H, Miura Y. Retinoid stimulates epidermal outgrowth of pig skin explants. J Dermatol (Tokyo) 1981.
20. Halprin KM, Taylor JR, Levine V, Woodyard C, Adachi K, Comerford M. Agents that activate cyclic AMP-dependent protein kinase inhibit explant culture growth and mitotic activity. J Invest Dermatol 1983; 81: 553–557.